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LETTER TO THE EDITOR

The Limit of PCR Amplification

Amplification of DNA fragments by the polymerase chain reaction (PCR) has significantly contributed to the rapid development of the modern biomedical sciences (Mullis *et al*., 1994). However, in spite of the tremendous amount of experimental work and the elegant description of PCR as a multitype branching-process (Nedelman *et al*., 1992), there is still some confusion about the number of different DNA molecules produced by the PCR. Here, we derive a simple formula for the number of PCR product DNA fragments, which represents the theoretical limit of the PCR amplification.

In most applications the substrate DNA molecule is very long and the two primers flank a short fragment (sDNA) to be amplified. From one very long DNA molecule the first cycle of PCR amplification produces two DNA molecules which have one long strand and one of medium length—we will refer to these DNA molecules as long DNA (1DNA). The second cycle of amplification produces four DNA molecules—two of which are 1DNA because they result from the primer extension of the two very long initial strands and the other two molecules consist of one strand of medium length and one strand of short length—we will refer to these DNA molecules as medium DNA (mDNA). The third cycle leads to a total of eight molecules: the two 1DNA molecules from the second cycle result in four DNA molecules already described for the second cycle; the two mDNA molecules regenerate two mDNA molecules, but also two new DNA molecules which consist of two short DNA fragments (sDNA)—this is the desired product of amplification and it appears for the first time in the third cycle. One may notice that the 1DNA molecules are used in each cycle and then regenerate themselves after the cycle. Therefore, after each cycle their number is constant and equal to two. The number of mDNA molecules, however, increases with each cycle because there are two sources of mDNA: (1) for each cycle two 1DNAs produce two mDNAs and (2) two mDNA molecules, produced from the 1DNA in the previous cycle, regenerate themselves to produce two mDNA molecules. Therefore, after each cycle the number of net resulting mDNA molecules increases by two molecules (two produced by the 1DNA + two regenerated—two used to regenerate the mDNA). Because the first cycle does not produce mDNA, the total number of mDNA molecules after n cycles is $2(n-1)$. After the third cycle of PCR amplification there are no new types of DNA molecules produced. Since there are only three types of DNA (1,m,s) and their total number after n cycles is 2^n (each subsequent cycle generates two-fold more molecules than the previous one), the number of sDNA molecules (N_{sDNA}), which are the product of interest, can be obtained by subtracting the number of 1DNAs (N_{1DNA}) and mDNAs (N_{mDNA}) from the total number of DNA molecules (N_{total}). Thus one obtains:

$$N_{sDNA} = N_{total} - N_{1DNA} - N_{mDNA}$$
$$= 2^n - 2 - 2(n-1) = 2^n - 2n.$$

The above expression was also derived by another method based on mathematical induction.

This formula indicates that initially the sDNA molecules are lacking or are a minority; however, at the fourth cycle ($n = 4$) the number of sDNA molecules ($=8$) equals the number of the other two types of DNA molecules (1DNA and mDNA). After the fourth cycle the number of sDNA molecules increases very rapidly (exponentially) while the number of medium fragments increases linearly with the cycle number. After the eighth cycle less than 10% of all DNA molecules are not sDNAs. After the 11th cycle the percentage of 1DNA and mDNA molecules decreases to less than 1%. Therefore, for all practical purposes the number of sDNA molecules can be approximated by 2^n after the 11th cycle. In some cases, where few PCR cycles are required, the quantitation should take into account the contribution of the 1DNAs and mDNAs, and the exact formula shown above must be used. This formula clarifies the theoretical question for the exact number

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of the PCR short DNA products and sets the theoretical limit of PCR amplification.

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